

### **REMARKS**

This is responsive to the final office action dated September 15, 2010. By this Response, Claim 48, 54, 55, and 75-77 are canceled, and Claims 56-64, 93, 100, 103-106, and 110-117 are pending for examination. Entry of this amendment is respectfully requested as it places the application in better condition for appeal. It is submitted that the rejections are overcome in view of the amendments and remarks presented herein, and that the application is in condition for allowance. Favorable reconsideration of the application is respectfully requested.

#### **I. REJECTIONS UNDER 35 U.S.C. §112**

The Final Office Action rejected claims 56-64, 93, 100, 103-106 and 110-117 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The Final Office Action stated:

Claims 56, 62, 93, 100 have been amended to recite a new limitation “wherein the transactivator is expressed at a level that could cause host cell death without an apoptosis protective protein . . . wherein the apoptosis protective protein prevents death of the host cell from the transactivator.” This new limitation is not supported by the specification as originally filed. The specification does not describe expressing the transactivator at a level that could cause cell death without an apoptosis protective protein, and does not describe such toxic expression level or range of such toxic level of said transactivator. Therefore, this newly added limitation constitutes new matter.

(9/15/2010 Final Office Action at pp. 7-8.)

Applicants respectfully traverse this rejection. As set out below, the specification of the patent application as filed provides written description support for the limitation “wherein the transactivator is expressed at a level that could cause host cell death without an apoptosis protective protein . . . wherein the apoptosis protective protein prevents death of the host cell from the transactivator” amended into Claims 56, 62, 93 and 100. The specification provides support for this claim language, including the following:

[0029] It is an object of the invention to provide methods and compositions for expressing a recombinant protein within a mammalian host cell using a co-expressed transcriptional activator. It is a further object of the invention to minimize or prevent adverse effects of the transactivator on host cell survival and proliferation.

(Specification at ¶ 29 (emphasis added).)

[0036] Another aspect of the invention provides methods of expressing or enhancing the yield of a desired recombinant protein in a mammalian host cell comprising introducing into the mammalian cell: (a) a first cistron encoding a transactivator under control of a first promoter, (b) a second cistron encoding an apoptosis-protective protein under the control of the first promoter or optionally under the control of a second promoter, and (c) a third cistron encoding a desired polypeptide under control of a third promoter, wherein said third promoter is responsive to the transactivator protein. Preferably, expression of the transactivator protein under control of the first promoter in the absence of the apoptosis-protective protein would cause significant cell death and expression of the apoptosis-protective protein prevents cell-killing due to expression of the transactivator. In one embodiment, the invention provides a method of linearizing a plasmid containing a transactivator protein and an apoptosis protective protein such that the apoptosis protective protein is transcribed upstream of the transactivator protein after integration into the host chromosome.

(Specification at ¶ 36 (emphasis added).)

[0039] Yet another aspect of the invention provides mammalian host cells comprising a first cistron encoding a transactivator, a second cistron encoding an apoptosis-protective protein that prevents cell-killing due to expression of the transactivator, and a third cistron encoding one or more desired proteins under the control of a promoter responsive to the transactivator. In a preferred embodiment, the transactivator is expressed from an efficient heterologous promoter at a level that, in the absence of the protective protein, causes significant cell death. In some embodiments, the invention provides a non-human mammalian host cell. An aspect of the invention also provides methods for producing a recombinant protein comprising culturing the mammalian host cells in a suitable medium such that the desired protein(s) is secreted into the medium.

(Specification at ¶ 39 (emphasis added).)

[0071] The term "apoptosis-protective protein," as used herein refers to a polypeptide product that, when expressed, decreases the frequency of transactivator mediated apoptosis in a population of cells.

(Specification at ¶ 71 (emphasis added).)

[0074] The invention provides improved methods for the activation of transcription from activatable cistrons by a transcriptional activator in a mammalian host cell that minimize or prevent adverse effects of the transactivator on cell growth and survival.

(Specification at ¶ 74 (emphasis added).)

[0076] Transactivators of the invention are polypeptides that enhance the expression of a desired gene by interacting directly or indirectly with nucleic acid sequences that are located in cis to the desired gene. The activator of the invention

is a protein that serves to activate transcription of an activatable cistron when expressed in a host cell but that shows adverse effects on host cell growth and survival when expressed in its normal or unmutated form at wildtype levels. In one embodiment, the transactivator of the invention is a protein that serves to activate transcription from an activatable control region in a host cell but that shows adverse effects on host cell growth and survival when expressed in its normal or unmutated form. The transactivators of the invention can be homologous or heterologous to the normal transactivator of the host cell. Examples of such transactivators include Ela proteins from adenoviruses and mammalian proteins that are components of signal transduction pathways, including mammalian p53, c-myc and cyclic-AMP response-element binding proteins (CREBs). Preferably, the homologous transactivator protein is derived from the host cell species. In one embodiment, the invention provides a hamster CREB derived from a hamster cell line.

(Specification at ¶ 76 (emphasis added).)

[0079] One embodiment of the invention provides methods of enhancing transcription of a cistron within a mammalian host cell comprising introducing into a mammalian host cell (a) a first cistron encoding a transactivator under control of a first promoter, (b) a second cistron encoding an apoptosis-protective protein, and (c) a third cistron encoding a desired polypeptide under control of a third promoter, wherein said third promoter is responsive to the transactivator protein. Preferably, expression of the transactivator protein under control of the first promoter in the absence of the apoptosis-protective protein would cause significant cell death and expression of the apoptosis-protective protein prevents cell-killing due to expression of the transactivator.

(Specification at ¶ 79 (emphasis added).)

[0080] The apoptosis-protective protein of the invention serves to prevent transactivator-mediated cell death.

(Specification at ¶ 80 (emphasis added).)

[0083] The combination of transactivator, protective protein, and activatable cistron in the same cell leads to significant increases in productivity of the activatable cistron. Preferably the specific production rate (production rate per cell) is enhanced at least two-fold by the combination of the transactivator and the protective protein. More preferably, the specific production rate is enhanced at least five-fold.

(Specification at ¶ 83.)

[0102] An aspect of the invention provides a mammalian host cell comprising a first cistron encoding a transactivator, a second cistron encoding an apoptosis-protective protein that prevents cell-killing due to expression of the transactivator, and a third cistron encoding one or more desired proteins under the control of a promoter responsive to the transactivator. In a preferred embodiment, the transactivator is expressed from an efficient heterologous promoter at a level at

which, in the absence of the protective protein, significant cell death would occur. Another aspect of the invention provides a mammalian host cell comprising a first cistron encoding a variant transactivator that retains transactivation activity but is defective in the ability to trigger apoptosis, and a second cistrons encoding one or more desired polypeptides under the control of a promoter responsive to the variant transactivator.

(Specification at ¶ 102 (emphasis added).)

“In order to satisfy the written description requirement, the disclosure as originally filed does not have to provide *in haec verba* support for the claimed subject matter at issue.” *Purdue Pharma L.P. v. Faulding Inc.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481 (Fed. Cir. 2000) (citing *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1570, 39 USPQ2d 1895 (Fed. Cir. 1996)). Nonetheless, “the disclosure of the prior application must ‘convey with reasonable clarity to those skilled in the art that, as of the filing date sought, [the inventor] was in possession of the invention.’” *PowerOasis, Inc. v. T-Mobile USA, Inc.*, 522 F.3d 1299, 1306, 86 USPQ2d 1385 (Fed. Cir. 2008).

The portions of the specification quoted above convey to the person of ordinary skill in the art that applicants were in possession of the inventions for recombinant protein expression recited in Claims 56-64, 93, 100, 103-106 and 110-117, including the claim limitation “wherein the transactivator is expressed at a level that could cause host cell death without an apoptosis protective protein . . . wherein the apoptosis protective protein prevents death of the host cell from the transactivator.” The above quoted sections from the specification also show that applicants did not add New Matter to Claims 56-64, 93, 100, 103-106 and 110-117 by amending into those claims the limitation “wherein the transactivator is expressed at a level that could cause host cell death without an apoptosis protective protein . . . wherein the apoptosis protective protein prevents death of the host cell from the transactivator.”

Thus, the rejection in the Final Office Action under 35 U.S.C. §112, first paragraph for lack of written description/claiming new matter is traversed, and Applicants respectfully request that this rejection be withdrawn.

## II. REJECTIONS UNDER 35 U.S.C. §103

Claims 48, 55-64, 75-77, 93, 100, 103-106, and 110-117 are rejected under 35 U.S.C. §103(a) as being unpatentable over Reff et al. (IDS), Cockett et al. (IDS) and Rao et al. (PNAS, 1992. Vol. 89, pages 7742-7746) in view of Antoniou et al. (WO 00/05393). The Applicants respectfully traverse these rejections because the cited prior art (1) fails to teach all the limitations of the claims, (2) teaches away from the invention as claimed, and (3) fails to provide a reasonable expectation of success.

Claims 48, 54, 55, and 75-77 have been canceled.

Independent Claims 56, 62, 93, and 100 claim host cells and methods of making recombinant protein where a transactivator for the desired protein is expressed at a level that can cause cell death, and this toxicity is countered by an apoptosis-protective protein that is expressed at levels to inhibit cell death caused by the transactivator. In addition, Claims 110-117 add limitations to these independent claims that require an increase in productivity of 2-5 fold from the transactivator and apoptosis-protective protein. These limitations are not disclosed in Reff, Cockett, Rao or Antoniou, and it would not be obvious to add these limitations to Reff, Cockett, Rao or Antoniou.

Reff teaches that apoptosis will naturally occur in the life cycle of host cells recombinantly expressing a desired polypeptide, when those host cells grow to a density such that the host cells are exposed to external factors such as nutrient limitation (e.g., serum deprivation), toxic agents in the growth media (e.g., waste products from cell growth), or physical stresses. (See Reff at ¶¶ 9, 13-14, 25-31, 34-35, 42-43, 76-87, and 98-123.) Reff demonstrates enhanced recombinant protein production (e.g., recombinant cultures can grow to a higher density where more cells are making the desired protein) when host cell life is extended beyond these limiting factors through the use of an apoptosis protective protein that prevents or delays apoptosis caused by these factors. (*Id.*) In view of the objects of the Reff invention, it would be detrimental to the host cell to add a toxic factor that would increase cell death

(apoptosis), such as the claimed toxic levels of a transactivator. Thus, it would not be obvious to obviate the objects of the Reff reference by combining it with toxic levels of transactivator protein as recited in the amended claims. Reff also provides no insights as to whether the claimed combination of toxic transactivator and rescuing apoptosis protective protein would enhance recombinant protein production as recited in Claims 110-117.

In addition, Reff teaches increased recombinant protein production by longer host cell lifetime (each cell produces for a longer time), and greater host cell density (the overall culture produces more protein per liter). In contrast, the pending claims enhance the amount of recombinant protein produced by increasing the rate of production per cell (each cell produces protein at a faster rate) using toxic levels of a transactivator combined with rescue through an apoptosis protective protein. This is a different way of enhancing recombinant protein production from Reff.

Cockett teaches that high levels of transactivator inhibited the growth of host cells. (See abstract.) Cockett obtained higher levels of recombinant protein using a weak promoter to express low levels E1A than with a strong promoter expressing high levels. (See Table 2.) Thus, Cockett teaches that high levels of transactivator are undesirable for recombinant protein expression.

Cockett attributes the poor performance with toxicity from the high levels of transactivator. This buttresses the teachings of Reff et al. that cell toxicity should be avoided because it is undesirable for recombinant protein expression. Cockett and Reff together teach that factors causing cell toxicity are to be avoided and this teaches away from the pending claims which intentionally introduce into the recombinant production system a factor causing toxicity. This teaching away is especially strong for toxic levels of transactivator that Cockett teaches are to be avoided.

At best, Reff combined with Cockett would teach that the host cells of Reff should be combined with low (non-toxic) levels of a transactivator to achieve recombinant protein expression. It would not be obvious from Cockett to modify Reff with toxic levels of a

transactivator and then rescue those cells with an apoptosis protective protein. Neither of these references teach this should be done or provide any basis to reasonably predict (or expect) what the impact of this toxicity and rescue would have on recombinant protein expression. Thus, Reff and Cockett, the two cited references relevant to recombinant protein expression, do not teach toxic levels of transactivator for inducing expression of desired protein, or rescuing recombinant production using an apoptosis protective protein, or a reasonable expectation of success for such recombinant protein expression under these conditions.

Since neither Reff nor Cockett teach toxic levels of transactivator for recombinant protein expression, or rescue with an apoptosis protective protein, the combination cannot teach the limitation of claims 110-117, that recombinant protein production is enhanced 2-5 fold by this combination of transactivator and apoptosis protective protein.

Rao does not overcome the shortcomings of Reff and Cockett because Rao does not teach recombinant protein expression. Instead, Rao teaches that primary cultures of rat kidney cells can be converted to a transformed cell type (a cancer like cell) by the E1A protein, but after an initial burst of growth many of these cancer-like cells die (by an apoptosis like process), and immortalized clones will arise from these E1A primary cells after 5-6 weeks. (See abstract, p. 7743, first col., first full paragraph.) Rao also teaches that during transformation the E1B protein can prevent the death of many of the E1A expressing cells after the initial burst of cell growth. (*Id.*) Thus, a person of skill in the art would not combine Rao (making cancer like cells) with Reff and Cockett (recombinant protein expression).

In addition, Rao does not teach the limitations of the claim which are missing from Reff and Cockett. Rao does not teach the use of a transactivator to induce recombinant protein expression, or use of toxic levels of transactivator to induce recombinant protein expression, or rescue of cells with such toxic levels of transactivator using an apoptosis protective protein during recombinant protein expression. Since Rao does not teach such recombinant production, Rao cannot teach enhancing recombinant protein expression 2-5 fold with this combination of toxic transactivator levels and rescue with an apoptosis protective protein. The combination of

these three references does not teach all the limitations of the pending claims, and so, the Applicants respectfully submit this rejection is overcome.

In addition, a person of skill in the art would not have a reasonable expectation of success for the claimed invention based on Rao, Reff and Cockett. Reff and Cockett do not provide a reasonable expectation of success (and in fact teach away) for the reasons stated above. Rao does not cure this defect. Rao was studying immortalization of primary cell cultures and not recombinant protein expression. Nothing in Rao teaches that E1A or E1B can be used for recombinant protein expression, or that the results with E1A and E1B in this cancer induction model has any relationship to recombinant protein production. No art has been cited to bridge this gap between recombinant protein expression as claimed in the pending claims and induction of a cancer like state in primary cells using E1A and E1 as taught by Rao. Thus, a person of skill in the art would not have a reasonable expectation of success based on the combination of Reff, Cockett and Rao, *i.e.*, that expression of toxic levels of transactivator with rescue by an apoptosis-protective protein would work for recombinant protein expression, or that such a combination would enhance recombinant protein expression 2-5 fold.

Antoniou was cited for teaching UCOE and IRES elements in an expression vector. These teachings do not bridge the gaps in Reff, Cockett and Rao, regarding expression of toxic levels of transactivator for inducing recombinant expression with rescue by an apoptosis-protective protein. Since Reff, Cockett and Rao do not teach such recombinant protein production, this combination cannot teach that such a combination will enhance recombinant production by 2-5 fold.

The case presented by the pending claims and Reff, Cockett and Rao is very similar to the Federal Circuit's decisions in *Eisai* and *Kinetic Concepts*. In *Eisai*, the Federal Circuit rejected an obviousness combination of prior art because the combination required that a feature the prior art found as advantageous be dropped to make the claimed invention. *See Eisai Co. Ltd. v. Dr. Reddy's Lab., Ltd.*, 87 USPQ2d 1452, 1456 (Fed. Cir. 2008) ("The record, however, shows no discernible reason for a skilled artisan to begin with lansoprazole only to drop the very

feature, the fluorinated substituent, that gave this advantageous property.”) As discussed above, Reff and Cockett together extol the benefits of reducing toxicity to host cells, including apoptosis, during recombinant production of desired proteins. Adding toxic levels of transactivator to Reff and Cockett would be contrary to the teachings of Reff and Cockett and would require that a person of skill in the art forego the advantageous feature taught by these references. *I.e.*, the object of the Reff publication is to reduce natural apoptosis and it would antithetical to that goal to artificially introduce to the host cell factors that would increase apoptosis over the natural factors. Cockett bolsters this by its teachings that toxic levels of transactivator are undesirable for recombinant protein expression. Combining Rao with Reff and Cockett causes the very incongruity relied upon by the Federal Circuit in *Eisai*, it requires that the person of skill introduce into Reff and Cockett the very thing these references teach should be avoided, toxicity to the host cell.

In *Kinetic Concepts*, none of the asserted prior art taught “treating a wound with negative pressure” and so the Federal Circuit affirmed the District Court’s holding of nonobviousness. *See Kinetic Concepts, Inc. v. Blue Sky Med. Grp, Inc.*, 554 F.3d 1010 (Fed. Cir. 2010); *see also, Honeywell Int’l, Inc. v. U.S.*, 93 USPQ2d 1740, 1747 (Fed. Cir. 2010) (prior art failed to teach perceptible red light as required by the claim). *Id.* The Federal Circuit held these claims nonobvious because the prior art failed to teach limitations of the claim. Similarly here, Reff, Cockett and Rao do not teach recombinant expression in a host cell using toxic levels of transactivator with rescue by an apoptosis protective protein. And, Reff Cockett and Rao do not teach that this combination increases recombinant protein production by 2-5 fold.

For all the above reasons, Applicant respectfully submits that the pending claims overcome the obviousness rejections combining Reff with Cockett, Rao and Antoniou.

### III. CONCLUSION

Applicants believe that this application is in condition for allowance, and request that the Examiner give the application favorable reconsideration and permit it to issue as a patent. If the Examiner believes that the application can be put in even better condition for allowance, the Examiner is invited to contact Applicant's representatives listed below.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 500417 and please credit any excess fees to such deposit account.

Respectfully submitted,

McDERMOTT WILL & EMERY LLP

/Andrew A. Kumamoto – Reg. No.: 40,690/

Andrew A. Kumamoto

Registration No. 40,690

18191 Von Karman Avenue, Suite 500  
Irvine, California 92612-7108  
Phone: 650.815.7437 AAK:pab  
Facsimile: 949.851.9348  
**Date: December 15, 2010**

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as our correspondence address.**